C O N T E N T S.

EXPERIMENTAL STUDIES ON SEX.

A STUDY OF ARGINASE CONTENT IN THE FOWL WITH SPECIAL REFERENCE TO SEX.

By A. C. CHAUDHURI, B.Sc.

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A STUDY OF ARGINASE CONTENT IN THE FOWL WITH SPECIAL REFERENCE TO SEX

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(*Received 5th June* 1927.)

The enzyme arginase which occurs in various organs of different animals was first discovered by Kossel and Dakin (1904) in the mammalian liver. Clementi (1916, 1918, 1922) found arginase in the liver of mammals, amphibians and fishes and the turtle, but he did not find any in the liver of birds and of the majority of reptiles. He found the enzyme to be present in the kidney of birds. Hunter and Dauphinee (1924) found a considerable amount of arginase in the liver of mammals and fishes. They also established the presence of arginase in the kidney of mammals and fishes, but usually in an amount smaller than that present in the liver. In the organs of fishes other than the liver, heart and kidney, the distribution of arginase appeared to be variable. In mammals these workers did not find arginase in any organs except the liver and the kidney, while in birds they could find it only in the kidney.

Edlbacher and Röthler (1925) made an extensive study of arginase content in the domestic fowl and in certain mammals. They found that arginase was present in the liver, kidney and testis of both mammals and birds. Further they detected the presence of the enzyme in the placenta and thymus of some mammals. These authors were the first to find any arginase in the testis, and they also found arginase in the liver of birds, which all the previous workers failed to detect. They established that in mammals the organ richest in arginase is the liver, while in birds it is the kidney. The relative quantity of the enzyme present per gram of body weight is far greater in mammals than in birds. They also demonstrated that the arginase value (which is the number of units of arginase per gram of body weight) was higher in the males than in the females.

The present piece of research was undertaken at the suggestion of Dr Crew, with the view to testing and extending the work of Edlbacher and Röthler. It is intended to present more data on the conflicting question of the presence and absence of arginase in the liver of birds and also to make a thorough study of its presence in different organs of the fowls, and its relation to sex.

The material used for the work consisted mainly of the Bantam breed of fowls. A few data refer to other breeds of fowls and to mammals.

The method used for detection and estimation of arginase throughout this experiment is that used by Edlbacher and Röthler, since it is the most up-to-date and claimed to be most accurate. Arginase can be extracted with water and dilute acetic acid, and can be separated from this solution by ammonium sulphate and alcohol and ether. It can also be extracted with glycerine. It is claimed that the

extraction with water is not so effective as that with glycerine. But so far as the organs of birds are concerned, where the quantity of arginase present is usually small, there was found to be little difference between the two methods of extraction. In quite a number of cases where control experiments were carried out with glycerine extract to test the presence of the enzyme where water extract failed to detect any, the results were again negative. Throughout this piece of work the extraction has been carried out with water. The one advantage this has over the method of glycerine extraction is that the organ can be used fresh, and can be handled with more accuracy. One gram of the organ to be tested was taken and ground with sufficient quartz-sand until a homogeneous paste was obtained. 25 c.c. of distilled water were then added, and the mixture well mixed; after standing for five minutes the extract was separated from the coarser sediment. A known quantity of this water extract was then taken for the determination of quantity of arginase present.

Edlbacher and Röthler found that the maximum activity was obtained when the reaction of the medium was 9·5 *p*H at a temperature of 38° C. For each estimation a definite quantity of 10 c.c. of the water extract was taken to which were added 5 c.c. of glycocoll-NaCl-NaOH of *p*H 9·5, then 10 c.c. of 1 per cent. arginine carbonate solution, and a few drops of toluol. This was corked and placed in a thermostat at 38° C. for one hour. At the end of one hour the ferment was destroyed by boiling for about 15 minutes. In each case control experiment without arginine carbonate was carried out. Next the solution was cooled, and the *p*H brought to 7 by the addition of phosphate buffer solution. The mixture was hydrolysed with urease (soya bean powder) and the evolved ammonium driven to a known volume of $N/50$ H_2SO_4 by passing a current of air through the apparatus. Finally the ammonium formed from the urea separated from arginine carbonate was estimated by titrating the excess of H_2SO_4 with $N/50$ NaOH. For a comparative study it is desirable that some definite unit be chosen for measuring the amount of the enzyme present—for this I have chosen the unit adopted by Edlbacher and Röthler. The unit of arginase is the quantity required to produce from 10 c.c. of a 1 per cent. arginine carbonate solution to which has been added 5 c.c. of glycocoll-NaCl-NaOH of *p*H 9·5 at 38° C. during the period of 60 minutes such an amount of urea which on being treated with urease produces 0·34 mg. of ammonium.

RESULTS.

In birds, kidney is the organ richest in arginase. This finding is in conformity with other workers. Next to the kidney, testis stands high for the presence of the enzyme. The exact amount varies in different individuals, but at the same time there is always a significant amount present in the materials examined. This finding is in accordance with the work of Edlbacher and Röthler but in contrast to that of Clementi, Hunter and Dauphinee. Clementi, Hunter and Dauphinee did not find any arginase in the liver of birds, while Edlbacher and Röthler found quite an appreciable amount. In my material the quantity present in the liver,

however, is smaller than in either the kidney or the testis. Out of the livers of 32 birds examined, only 11 showed any trace of the enzyme, and the amount present was very small. It may be that the enzyme is not universally present in the liver of birds, or that the very small quantity which is usually present has escaped extraction in the above 21 out of 32 cases.

REPRODUCTIVE ORGANS.

As was mentioned above, there is a certain amount of arginase present in the testis and also in the vas deferens. No arginase was ever found in the ovary, oviduct, or in the ova. This indicates that the presence of arginase in the reproductive organs is a sex-dimorphic character.

OTHER ORGANS.

Several determinations with thyroid, thymus, heart, spleen, pancreas and blood of fowls did not reveal the presence of any traces of arginase. The organs of 17 birds were examined fully for quantitative estimation of the enzyme, and the results obtained have been tabulated in Table I.

Table I (*a*).

No.	Breed and Sex	Body wt. in gm.	Units of arginase per gm. of kidney	Total no. of units in kidney	Units of arginase per gm. of testis	Total no. of units in testis	Units of arginase per gm. of liver	Total no. of units in liver	Total units	Units per gm. of body wt.
1	Bantam ♂	490	17·625	68·032	2·625	18·90	0·75	8·25	95·18	0·194
2	,,	504	9·00	57·30	3·00	15·27	0	0	57·30	0·113
3	,,	518	25·125	82·912	6·625	51·41	0	0	134·32	0·259
4	,,	560	15·75	64·10	0·75	3·27	0	0	67·37	0·120
5	,,	588	11·50	55·08	1·50	9·91	0	0	65·00	0·110
6	,,	672	9·25	44·40	1·00	8·50	0	0	52·90	0·078
7	,,	686	7·75	53·63	2·75	23·18	0	0	71·41	0·112
8	,,	756	22·125	139·387	4·00	23·52	0·75	15·50	178·41	0·235
9	Ancona ♂	1876	22·75	286·65	2·25	21·01	0	0	307·66	0·163
Average ♂			15·65		2·72					0·153

(*b*)

No.	Breed and Sex	Body wt. in gm.	Units of arginase per gm. of kidney	Total no. of units in kidney	Units of arginase per gm. of liver	Total no. of units in liver	Total units	Units per gm. of body wt.
1	Bantam ♀	448	6·50	28·405	1·50	16·65	45·05	0·100
2	,,	462	6·75	30·71	0	0	30·71	0·066
3	,,	462	9·50	40·37	0·25	3·86	44·23	0·095
4	,,	504	13·00	55·90	0	0	55·90	0·110
5	,,	504	18·00	80·28	0	0	80·28	0·159
6	,,	518	17·50	88·37	0	0	88·37	0·170
7	,,	518	12·25	45·32	0	0	45·32	0·087
8	,,	532	10·25	40·48	0·25	3·79	44·28	0·083
Average ♀			11·71					0·107

The results obtained have been divided into two sections (*a*) and (*b*) according to the sex of the birds. In both the sections, the figures have been arranged according to the ascending order of the body weight of the individuals. It is evident from the figures that the average arginase value of the males is higher than that of the females. The value for females (0·107) is 69·9 per cent. of that of males (0·153). The average number of units per gram of the kidney of the males, which is 15·65, is also higher than that of the females, which is 11·71. This latter is 74·8 per cent. of the former. From the above figures it is proved that there is a sex-dimorphism with regard to arginase content in fowls, and that the higher arginase value is associated with maleness.

ORIGIN OF THE ENZYME.

It has been shown that there is no arginase in the ovary or in the mature ova, but there is a significant amount in the testis and vas deferens. The question then arises how the new individual comes to possess the enzyme. It is probable that the fertilised ovum gets its arginase from the male through the spermatozoa. A few eggs were examined which showed no trace of the presence of the enzyme. This is perhaps due to the small amount of the enzyme introduced by the spermatozoa. A few newly hatched chicks were examined and showed the presence of a significant amount of the enzyme. Three chicks were studied for quantitative estimation; the results obtained are given below. The testes, owing to their small size, were not examined.

Table II.

No.	Newly hatched chick. Sex	Body wt. in gm.	Wt. of kidney in gm.	Total units	Units per gm. of body wt.
1	♂♀♂♀	38·0	0·32	0·75	0·019
2		33·5	0·25	2·50	0·074
3		42·0	0·40	2·00	0·047
Average					0·046

It is seen that the enzyme arginase which was not detectable in the unincubated eggs can be estimated as soon as the eggs are hatched. Clementi found arginase in human embryo as early as four months. It would be of interest to determine after how many days of incubation the eggs show an appreciable amount of the enzyme and also whether it has any relation to the time of sex-differentiation.

Apart from the fowl material, only a few specimens of liver and testis of mammals were examined. With regard to the testis of a cat and a rabbit, it can be stated that both exhibited the presence of arginase which is contrary to the finding of Hunter and Dauphinee, but in agreement with those of Edlbacher and Röthler.

As regards the function of the enzyme, very little is as yet known except that it hydrolyses arginine into ornithine and urea. As the result of his researches, Clementi, who did not find any arginase in liver of birds, but found it in the

liver of mammals, amphibians and fishes, enunciated the doctrine that arginase is present in the liver of all those animals which have what he calls a "ureotelic" metabolism, that is in which urea is the final product of protein degradation, and absent from the liver of those in which protein metabolism is "uricotelic"—ending in uric acid. Since the work of Edlbacher as well as the results of the present investigation do not show that arginase is totally absent from the liver of birds, it is rather difficult to accept his doctrine.

I have great pleasure in thanking Dr Crew for the help and encouragement he has given during the course of this study.

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XII.—The Erythrocyte Count in Sexually Normal and Abnormal Fowls.

By A. C. Chaudhuri, Animal Breeding Research Department, University of Edinburgh.

(Read 20th December 1926. Received 20th December 1926.)

FISH and Hayden (1926) have presented certain data of their own and of previous investigators (Welsch, 1923; Sustchowa, 1910) concerning the erythrocyte count in the sexually normal and abnormal pig, sheep, and goat. Other data recorded by Makotine (1910) relating to the horse, cat, and sheep are referred to in a paper by Blacher (1926), which deals mainly with the fowl. In view of Blacher's findings, Dr Crew suggested that it would be profitable to examine the erythrocyte count in certain fowls, which, having once functioned as hens, had undergone complete sex-transformation to become fecund and potent cocks.

The figures given by Blacher are as follows:—

TABLE I.

Sex.	No.	Maximum.	Minimum.	Average.
Normal ♂♂	26	4,440,000	3,060,000	3,772,000
Normal ♀♀	33	3,688,000	2,116,000	2,870,200
Castrated ♂♂ . . .	9	3,010,000	2,000,000	2,716,000
Ovariotomised ♀♀ . .	5	2,940,000	2,460,000	2,822,000
Incomplete ♂	1	...	...	3,340,600
Feminised ♂ (Ovary + Testis)	1	...	...	2,860,000

Blacher concludes that the number of erythrocytes (and also the quantity of hæmoglobin) is lower in the hen than in the cock: that after gonadectomy the number falls in the male, whereas in the case of the female there is no significant change; and that climatic variations and differences in husbandry can be responsible for a considerable variability in the erythrocyte count.

In order to establish standards with which the sexually abnormal birds might be compared, the following data were secured. The enumeration was made by the use of the Thoma Zeiss hæmocytometer, the blood being diluted 1 in 200.

TABLE II.

Adults.

♂ ♂			♀ ♀		
No.	Breed.	Count per c.mm.	No.	Breed.	Count per c.mm.
1	F_1 Light Sussex ♀ × Red Sussex ♂	4,448,000	1	Rhode Island Red .	3,568,000
2	,, ,,	4,832,000	2	,, ,, .	3,032,100
3	,, ,,	4,160,000	3	,, ,, .	2,968,000
4	,, ,,	4,632,000	4	,, ,, .	2,072,000
5	,, ,,	3,968,000	5	Barred Rock . .	3,456,000
6	,, ,,	4,656,000	6	,, . .	3,072,000
7	,, ,,	4,760,000	7	,, . .	3,336,000
8	,, ,,	5,200,000	8	White Leghorn .	2,696,000
9	,, ,,	4,544,000	9	,, ,, .	3,832,000
10	,, ,,	4,472,000	10	Sussex Cross . .	2,896,000
11	,, ,,	4,080,000	11	,, . .	3,336,000
12	,, ,,	4,544,000	12	,, . .	3,256,000
13	Campine . .	4,984,000			
Highest . . .		5,200,000	Highest . . .		3,832,000
Lowest . . .		3,968,000	Lowest . . .		2,072,000
Average . . .		4,560,000	Average . . .		3,127,000

The mean count, in thousands, of the normal male is 4,560 ± 63; that of the normal female 3,127 ± 85. The difference is 1,433 ± 106, being 13·5 times its probable error, and therefore significant. This finding is in general agreement with that of Blacher.

In order to find out whether or not bantams exhibited this sex dimorphism, the following data were collected:—

TABLE III.

Bantams.

♂ ♂			♀ ♀		
No.	Breed.	Count per c.mm.	No.	Breed.	Count per c.mm.
1	Old English Game .	5,512,000	1	Old English Game .	3,784,000
2	,, ,, .	5,112,000	2	,, ,, .	3,400,000
3	,, ,, .	5,280,000	3	,, ,, .	3,600,000
4	,, ,, .	4,968,000			
Average . . .		5,218,000	Average . . .		3,594,000

It is seen that this sex-dimorphism is evidenced.

In order to find out whether or not this sex-dimorphism was exhibited by sexually immature fowls, the following data were collected:—

TABLE IV.

♂ ♂			♀ ♀		
No.	Breed.	Count per c.mm.	No.	Breed.	Count per c.mm.
1	White Leghorn .	3,296,000	1	Barred Rock . .	3,696,000
2	,, ,, .	4,048,000	2	,, ,, . .	3,744,000
3	,, ,, .	3,936,000	3	,, ,, . .	3,296,000
4	,, ,, .	2,968,000	4	,, ,, . .	4,296,000
5	,, ,, .	3,168,000	5	,, ,, . .	3,600,000
6	,, ,, .	4,072,000	6	,, ,, . .	4,368,000
7	Barred Rock . .	3,736,000	7	,, ,, . .	3,136,000
			8	,, ,, . .	3,112,000
Average . . .		3,603,000	Average . . .		3,656,000

It is seen that there is no difference in the matter of erythrocyte number between sexually immature male and female fowls.

Having thus established standards for comparison, it was possible to proceed to the examination of certain classes of sexually abnormal birds.

TABLE V.

No.	Breed.	Average Number of Erythrocytes per c.mm. in two determinations at a Week's Interval.
	(a) *Cases of Complete Sex-Reversal.*	
1	White Leghorn	5,672,000
2	Ancona × Rhode Island Red .	4,636,000
3	Wyandotte	3,384,000
4	Leghorn × Wyandotte . .	4,744,000
5	Brown Leghorn	5,244,000
6	,, ,, ...	3,656,000
	Average	4,556,000
	(b) *Cases of Incomplete Sex-Reversal.*	
7	Light Sussex	5,052,000
8	Rhode Island Red . . .	3,872,000
9	Houdan × Leghorn . . .	4,700,000
10	White Leghorn	3,164,000
11	Rhode Island Red . . .	4,420,000
12	White Leghorn	4,272,000
13	,, ,, ...	4,644,000
14	,, ,, ...	4,440,000
	Average	4,320,500

The average of these abnormal birds is 4,421,000, a figure much nearer that of the normal male than that of the normal female.

The following experimental birds, cockerels and pullets, were next examined (only one determination was made).

TABLE VI.

No.	Description of Bird.	Count per c.mm.
1	Brown Leghorn ♂ with implanted testis from Silver Campine	3,496,000
2	Do. do. do.	4,000,000
3	Silver Campine ♂ castrated . . .	3,184,000
4	Silver Campine ♂ with implanted ovarian tissue	3,000,000
5	Brown Leghorn ♀ with implanted testis .	3,044,000
6	Silver Campine ♀ with implanted testis .	4,424,000
7	Brown Leghorn ♀ with extra ovarian tissue implanted	2,480,000
8	Do. do. do.	2,784,000

There is nothing very significant in these figures. It is worthy of note, however, that the count in the female with extra ovarian tissue is distinctly lower than the female, though not significantly so since it comes within the range exhibited by the normals, and also, as was noted by Blacher, that in the castrated male the number is lower than that of the normal male.

I wish to express my gratitude to Dr Crew for the help which he has given to me during the course of this study.

Summary.

1. The number of erythrocytes in a unit volume of blood is significantly higher in the sexually normal adult male than in the normal adult female of the fowl.

2. This difference in erythrocyte count is not exhibited by sexually immature fowls. In cases of sex-reversal, the number is much nearer that of the normal male than that of the normal female.

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THE EFFECT of SUB-CUTANEOUS INJECTION of ALCOHOL into the MALE MOUSE on the SEX-RATIO of the OFFSPRING.

BY

AMAL CHANDRA CHAUDHURI, B.Sc.,

THE EFFECT of SUB-CUTANEOUS INJECTION of ALCOHOL into the MALE MOUSE on the SEX-RATIO of the OFFSPRING.*

I N T R O D U C T I O N

It seems a debatable question whether there is a differential effect of alcohol on male and female producing spermatozoa when the males are treated with alcohol.

STOCKARD & PAPANICOLAON found an abnormal sex-ratio from guinea-pigs where the parent and ancestors were treated with alcohol fume. BLUHM treated male mice with ethyl-alcohol by injection method and found a rise in the resulting sex-ratio.

On the other hand, MACDOWELL & LORD treated mice with alcohol by fumigation method, but found no difference in the sex-ratio as compared with the normal. It has been shown that the germ cell can be affected by alcohol - such as treating guinea-pigs with alcohol fume. STOCKARD & PAPANICOLAON found more pre-natal mortality and more abnormal progeny and reduction in the litter size. Working with

fowls/

* A paper on this subject has been accepted for publication in the British Journal of Experimental Biology.

fowls PEARL found reduction in'fertility', and his suggestion on the effect of alcohol on germ cell, is that alcohol and similar substances act as selective agents upon the germ cells of treated animals. MACDOWELL (1922) treated white rats with fumes of alcohol and found a reduction in fertility.

Considering the effect of alcohol on fertility in the above cases, it is quite clear that the alcohol fume acts as a selective agent on the germ cells by eliminating the less resistant ones. It is probable also, that alcohol has the effect of reducing the activity of the germ cell.

At this stage reference might be made to the interesting work of COLE & DAVIS (1914) with rabbits. They found that when two male rabbits were mated to one female, superfoetation occurred, and part of the resulting litter of young were sired by one male or the other. One male was found to have sired the majority of young of a given litter, and in the total number of competition matings. This male with the fertilising advantage was then treated with alcohol by the inhalation method. As a result of this treatment his sperms became affected in such a way that mated in competition with the same male he normally had beaten, he now failed to sire any young, yet when/

when mated singly or alone with a female, he still possessed the power to beget offspring.. This experiment shows how a short alcohol treatment may weaken the fertilising ability of spermatozoa.

In the light of this experiment, one is led to think whether x and y spermatozoa would not be differently affected. The result of the previous work in this line is conflicting, so the object of this paper is to present the result of an experiment which was carried out to throw more light on the subject.

MATERIAL

The experimental animals used were Albino Mice. The strain was from a stock kept in the Department and then bred. The data covers the sex record for 740 individuals, of which 336 came from matings where the father had been treated with alcohol and 404 came from normal matings.

METHOD.

The individual mice selected for each group was by random method, but care was taken that too/

too many younger or older mice were not included in one group. The mice were bred in small boxes. In each breeding box 2 males and 3 to 4 females were kept, In the third week of pregnancy each female was removed to smaller, separate boxes till parturition. This procedure was taken to prevent the litters being eaten by other mice, and also to prevent the mixing up of two or more litters. After parturition, each litter sexed and the mother returned to the breeding box and re-mated. As regards sexing, each litter first sexed on external characters, and then most of them confirmed by examining the gonads after dissection. It might be stated that there is no error in the data due to sexing.

In the case of the experimental group the males were treated with alcohol. The mode of treatment was by sub-cutaneous injection on the back. The alcohol used was 20% ethyl alcohol. The dose applied was ·2cc. to start with, then it was increased to ·25 cc. A dose of ·25-30% alcohol proved fatal. The males in the treated group were injected on alternate days.

With a little practice it was quite easy to do the injection. A folded handkerchief was wrapped round/

round the mouse, and it was held by the tail with the left hand, thus leaving the right hand free to do the injection.

RESULTS.

Although it is not the purpose of this paper to study the effect of alcohol so treated, on the individuals themselves, yet one or two words might be said about the behaviour of the individuals after treatment. The effect produced after injection is somewhat like the effect on the guinea-pigs as noted by STOCKARD & PAPANICOLAON, when the dose of alcohol was given through the mouth to the stomach but it was not so acute. The mice showed signs of intoxication, but it was not a complete stupor. They were intoxicated quite a long time. Again the intoxication was not so acute in the method by injection, as it was when a mouse was treated with fumes of alcohol for an hour. According to STOCKARD the effect of inhalation is a short acute effect - while alcohol in the stomach is gradually and continuously absorbed for a considerable length of time, so that the animal tissue is acted upon for hours after receiving the dose./

dose. The same may be said for the injection method. After the injection the liquid is gradually absorbed into the body. The object of treatment being to affect the germ cells if possible, so the longer the effect remains the greater is the possibility. This experiment has been directed to test whether the treatment would have a different effect on the x and y_A bearing spermatozoa. If there is any difference then it may be possible to detect this by examining the resulting sex-ratio. If there is a significant difference in the sex-ratio so obtained, from that of a normal control, where the males have not been treated, then it is sure that either the one sex or the other is suffering. This might be due to:-

(1)Excessive pre-natal mortality falling on one sex - or

(2)During fertilisation one sex suffers some sort of disadvantage.

As regards the first cause, MACDOWELL & LORD (1926) have shown that in the mouse there is no continuous selective elimination of one sex or the other before birth. As regards the second reason, if one of the two kinds of spermatazoa (x or y) are affected more, then the sex fertilised by that kind of spermatozoa will suffer.

If there is any variation in the sex ratio from/

from the treated males , then it may probably be due to the latter cause.

The results obtained from such matings are tabulated along with the normal controls in TABLE I.

TABLE/

TABLE I

1. TREATED MALE x UNTREATED ♀

WHEN BORN	TOTAL NO. YOUNG	NO. LITTERS	♂♂	♀♀	SEX-RATIO	PER-CENTAGE ♂
March	44	6	27	17	158.82	61.36
April	48	7	25	23	108.69	52.08
May	108	17	57	51	111.76	52.77
June	104	17	54	50	108.00	51.92
July	32	4	21	11	190.9	65.62
TOTAL	336	51	184	152	121.05	54.76 ± 1.82

2. UNTREATED ♂ x UNTREATED ♀

February	82	15	39	43	90.69	47.56
March	48	7	22	26	84.61	45.83
April	14	2	7	7	100.00	50.00
May	68	10	29	39	74.35	42.64
June	70	10	29	41	70.73	41.42
July	29	4	8	21	38.09	27.58
August	93	14	39	54	72.22	41.93
TOTAL	404	62	173	231	74.89	42.82 ± 1.65

The number of litters obtained from both the experimental and the control groups have been arranged according to the month in which they were born. The first part of TABLE I. gives data of the alcoholic group, the second part gives that of the normal. The sex-ratio and the percentage of males for each month, in the first part of the Table are higher than those in the second part of the Table. The mean percentage of ♂'s with its probable error in the alcoholic group is 54·76±1·82 and the mean percentage of ♂'s with its probable error in the normal group is 42·82± 1·65. The difference with its probable error is 11·94±2·45 showing in favour of the alcoholic group. The difference is about five times its own probable error which appears to be statistically significant.

The probable errors for the above were calculated from the formula $0{\cdot}6745\ \frac{P.Q}{N}$ as used by PEARL(1917).

The normal sex-ratio in mice by SCHULTZE, as mentioned by PARKES is approximately equal, while COPEMAN & PARSON note the number of ♂'s is slightly in excess of the ♀'s. PARKES found a total sex-ratio of 118·♂ per 100 ♀'s. BLUHM found 79·30 per 100 ♀'s for his controls. My own figure for/

for the normal is 74·8, which is a low figure compared with the figures mentioned above. This low figure might be due to a certain extent to the influence of the breeding season as observed by PARKES. According to his Table I. (p.326) he gets 49·7±1·78% ♂'s from March to June as compared to 56·5±1·27% from July to October.

DISCUSSION.

TABLE I. shows that the treatment of alcohol raises the sex-ratio in mice. This might quite possibly be due to alcohol having a weakening effect on female producing spermatozoa, thus giving a better chance to the male producing spermatozoa in the competition to fertilise the ova.

BLUHM also found that the sex-ratio was raised in mice when the males were treated with alcohol by injection. She found 56·2% ♂'s from treated ♂'s while 48·5% ♂'s from untreated males.

STOCKARD & PAPANICOLAON'S work on guinea-pigs with alcohol by the fumigation method, confirms that the treatment of parents and ancestors with alcohol has a disturbing effect on the sex-ratio/

ratio. They found that when the male parent or male and female parents, or male ancestors or male and female ancestors were treated, the sex-ratio was raised, but it was lowered when the female parent or female ancestors only were treated.

MACDOWELL & LORD treated mice with alcohol fumes, but found no variation in the sex-ratio.

PEARL treating the domestic fowl with alcohol fumes, found no significant difference in the sexes produced in the alcoholic and normal control series.

Recently DANFORTH (1926) found that the sex-ratio was raised by treating the male mice with ethyl alcohol fumes. The mice were treated twice a day at least for one hour. He found a sex-ratio 128, from those treated, as against 103·2 from those untreated.

HANSON & HEYS (1925) treated Albino rats for ten generations, rising out of a single pair, and conclude that alcohol does not modify the sex-ratio. But they get a difference of $3·72 \pm 1·21$ in the totals showing in favour of the treated group. They get a difference for 7 out of 9 generations in favour ofthe treated group, but the probable errors are high which is greatly dependent on the numbers involved.

CREW/

CREW (1926) treated Albino male mice with fumes of alcohol, but no significant disturbance of the sex-ratio was observed.

Considering all the cases stated above, it is difficult to say why in some cases alcohol is found to modify the sex-ratio, while in others it seems to have no effect. It is probable that alcohol is effective in modifying the sex-ratio if the mating takes place when there is still some alcohol in the animal's tissue, or perhaps if the dose be severe enough.

CONCLUSION.

It is possible to modify the sex-ratio in mice by injecting ethyl alcohol sub-cutaneously into the back of males prior to breeding. The work is at least in keeping with some workers on the subject.

I am greatly indebted to Dr.F.A.E.Crew for suggesting this piece of work, and for the advice and encouragement he gave me.

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THE IODINE CONTENT of the THYROID of the FOWL with REFERENCE to AGE and SEX.*

by

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The IODINE CONTENT of the THYROID of the FOWL WITH REFERENCE to AGE and SEX.

The iodine content of the thyroids of certain animals, as estimated by previous workers on this subject, is shown in the following table :-

TABLE I

SPECIES	PERCENTAGE OF IODINE OF DESICCATED THYROIDS.			AUTHORITY.
	Maximum	Minimum	Average	
Man	0.588	0.051	0.349	Seidell.
Dog	0.358	0.131	0.220	Seidell.
			0.332	Marine and Lenhart.
	0.265	0.011	0.095	Cameron.
Sheep	0.283	0.032	0.166	Seidell.
			0.246	Marine and Lenhart.
Cattle	-	-	0.346	Marine and Lenhart.
Pig	-	-	0.251	Marine and Lenhart.
Pigeon	-	-	0.485	Cameron.

It is noticed that among these figures there is considerable disparity. Some is doubtless due to/

to errors and differences in methods of investigation. Much, no doubt, is the reflection of the facts that the iodine content is an indication of the relative functional condition of the thyroid and that the activity of the thyroid varies with the season, with the phases of the reproductive cycle, with health, with diet, and so forth. It is seen that in the list the iodine content of the thyroid of the bird is greater than in the case of the mammal.

The thyroids of healthy, freshly killed animals were carefully and cleanly removed, and thoroughly desiccated over concentrated H_2SO_4. When perfectly dry, the tissue was accurately weighed. In the case of the fowl the total thyroid tissue of each individual was used. Kendall's method for the estimation of iodine was employed. In order to obviate any variation in iodine content due to season (Marine and Lenhart, 1922), this study was completed during the spring.

I

THE IODINE CONTENT of SEXUALLY IMMATURE and MATURE FOWLS.

Twelve/

Twelve white Wyandotte cockerels from the same parental pen, of the same age, and all kept under identical conditions, were used. The onset of sexual maturity was determined by histological examination of the testes. It was found that spermatogenesis was active and complete at 14 weeks.

TABLE/

TABLE II

No. of BIRD.	Age in WEEKS WHEN KILLED	BODY WEIGHT in GRAMS	WEIGHT of DESICCATED THYROID in GRAMS.	TOTAL IODINE in MILLIGRAMS	PERCENTAGE of IODINE of DESICCATED THYROID
1	7	350	0.007	0.0140	0.200
2	8	448	0.007	0.0306	0.437
3	8	-	0.007	0.0201	0.287
4	9	-	0.006	0.0227	0.379
5	9½	518	0.007	0.0254	0.363
6	10	420	0.004	0.0140	0.350
7	12	672	0.010	0.0422	0.422
8	13½	1,036	0.012	0.0315	0.262
9	14	770	0.010	0.0520	0.520
10	14	840	0.015	0.0780	0.520
11	14½	1,092	0.013	0.0736	0.566
12	15	980	0.014	0.0672	0.480

It is seen that at 14 weeks, that is, at the time of sexual maturity, in this stock, the iodine content becomes higher. The suggestion which emerges from these figures is that the iodine content is higher in the sexually mature bird, the increase being associated with the establishment of active spermatogenesis. In the case of the dog, MARINE and WILLIAMS (1908) record a low iodine content in individuals under 6 months old.

II

THE IODINE CONTENT and SEX.

FENGER (1912-13) in the case of cattle foetuses, found that the iodine content of the female thyroid was higher than that of the male and suggested, therefore, that the female thyroid was functionally more active than that of the male foetus of the same age. In order to examine this question of a possible sex-dimorphism of this kind in the fowl, 14 adult bantams (Old English Game) were examined.

TABLE/

TABLE III

NO.	SEX	WEIGHT OF DESICCATED THYROID IN GRAMS	TOTAL IODINE IN MILLIGRAMS	PERCENTAGE OF IODINE OF THE DESICCATED THYROID
1	♂	0.009	0.0405	0.450
2	♂	0.020	0.2200	1.100
3	♂	0.012	0.0680	0.566
4	♂	0.009	0.0585	0.650
5	♂	0.012	0.0748	0.623
6	♂	0.010	0.0583	0.583
7	♂	0.012	0.0669	0.557
Average	♂			0.647 ± 0.049
1	♀	0.010	0.0360	0.360
2	♀	0.012	0.0990	0.825
3	♀	0.012	0.077	0.641
4	♀	0.010	0.0496	0.496
5	♀	0.012	0.0637	0.530
6	♀	0.015	0.0863	0.575
7	♀	0.010	0.0520	0.520
Average	♀			0.563 ± 0.033

The difference is 0.084 ± 0.0578 which is 1.4 times its own probable error, and is statistically negligible.

These/

These figures afford no evidence that in the case of these fowls a sex-dimorphism in the matter of iodine content of the thyroid exists.

III.

THE IODINE CONTENT of the THYROIDS of MAMMALS and BIRDS COMPARED.

In order to examine anew the suggestion which appeared in TABLE I, that the iodine content of the bird was higher than that of the mammal, I made use of whatever material became available and the result of the examination of these specimens is given in the following table. In the case of the larger mammals only a portion of the thyroid was used.

TABLE IV/

TABLE IV.

NO.	ANIMAL	WEIGHT OF DESICCATED THYROID IN GRAMS	TOTAL IODINE IN MILLIGRAMS	PERCENTAGE OF IODINE IN DESICCATED THYROID	AVERAGE PERCENTAGE OF IODINE OF THE SPECIES.	AVERAGE PERCENTAGE OF IODINE OF THE GROUP
18	Dog	0.200	0.4928	0.246	0.357	
24	Dog	0.205	0.9600	0.468		
20	Goat	(0.1)	(0.187)	0.187	0.222	0.215 ± 0.031
27	Goat	0.240	0.6210	0.258		
21	Rabbit	0.065	0.0340	0.052	0.116	
22	Rabbit	0.054	0.0930	0.173		
23	Rabbit	0.029	0.0350	0.123		
25	Duck	0.020	0.1240	0.621	0.862	
26	Duck	0.030	0.3310	1.104		
4	Fowl	0.035	0.4128	1.179		
5	Fowl	0.045	0.4704	1.045		0.697 ± 0.033
6	Fowl	0.060	0.4896	0.816	0.887	
10	Fowl	0.030	0.2113	0.704		
19	Fowl	0.020	0.1386	0.693		
Average of 14 bantam fowls (from TABLE III)					0.605	

The difference is 0.482 ± 0.0451 which is 10.7 its own probable error and therefore significant.

From these figures it would appear to be the case that the iodine content of the thyroid of the bird is indeed higher than that of the thyroid of the mammal. It did not seem reasonable to ascribe any significant part of this difference to differences in reproductive activity, in food, or in general health, and all were adult individuals.

I wish to express my thanks to DR. F.A.E. CREW, who called my attention to the possible interest of this study, for his help during its course.

SUMMARY/

S U M M A R Y.

1. The iodine content of the thyroid of the sexually immature male fowl is lower than that of the thyroid of the sexually mature. The increase in iodine content is coincident with the attainment of sexual maturity as estimated by the histological picture of the testis.

2. In the case of the material used there was no indication of a sex-dimorphism in the matter of iodine content.

3. The average percentage of iodine content of the thyroid of the bird is significantly higher than that of the mammal.

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AN EXPERIMENTAL STUDY ON THE POSSIBLE RELATION OF METABOLIC RATE TO SEXUAL DIFFERENTIATION IN THE FOWL.

by

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I N T R O D U C T I O N.

The work of HERTWIG (1912) on the frog has shown that "over-ripening" of the eggs leads to an effective modification of the sex-ratio in the direction of an increased production of males.

ADLER (1920) examining the thyroids of the larvae arising from such "over-ripe" fertilized eggs found that they are larger than in normal individuals, and that the differentiation of the thyroid gland occurs at an earlier stage in development than that of the gonad. From this study the hypothesis was formulated that the larger and hyperfunctioning thyroid had influenced the processes of sexual differentiation of the gonad of the genetic female in the male direction.

RIDDLE (1914-1916) and WHITMAN (1919) working with the pigeon demonstrated that from birds made to produce many eggs during the reproductive season there arise more males during the early part (Spring) and more females later. The male-producing eggs of the early season were found to be smaller than the female-producing eggs elaborated later in the Summer. RIDDLE regards the male-producing ova of the Spring as ova exhibiting a relatively high metabolic rate and female-producing ova of the later season as cells of a lower metabolic rate.

RIDDLE & FISHER (1925) in a more recent paper have shown that the period of most abundant male pro-

duction is the phase of the reproductive season characterized by the largest thyroid size in the parent and these authors conclude that successful modifications of sex-differentiation have been effected through changes in the rate of metabolism in the ova and early embryos.

The following experiments were designed with a view to determining whether or not an elevation of the metabolic rate in the chicken embryo, at a stage in its development before the visible differentiation of the reproductive organs has occurred, would result in a modification of the processes of sexual differentiation.

TECHNIQUE.

WILLIER (1924) by grafting thyroid tissue on to the chorioallantoic membrane of the developing chick embryo obtained a number of dwarfed specimens. The small size and the emaciated condition of these embryos were regarded as signs of hyper-thyroidism resulting in an increased metabolism. In these experiments, however, it was found that the earliest favourable time for the implantation of the thyroid tissue was the ninth or tenth day of incubation, at a stage, that is, when the sexual differentiation of the gonads had already occurred. To obviate this difficulty the following procedure was adopted in this series of experiments.

At the third day of incubation (i.e., at a stage in development when no demonstrable differentiation of the gonads into testis or ovary has occurred) a preparation of the active principle of the thyroid gland (thyroxin) dissolved in saline was injected into the air space of the egg. An alternative method of injecting the thyroxin directly into the albumen was tried, but was found to be unsatisfactory as the increased local pressure produced numerous cracks in the shell radiating from the aperture through which the fluid was injected.

The thyroid preparation used was the mono-sodium salt of thyroxin prepared by the British Drug Houses.

After removal of the egg to be injected from the incubator, the broad end of the shell was rubbed with a pad soaked in 70% alcohol, then by means of a fine dental burr a small hole was drilled in the shell in a line at right angles to the long axis of the egg and at a point about one centimetre from the apex. The solution of thyroxin was then introduced into the air cavity by means of a hypodermic syringe with a fine, slightly curved needle. If the air space had been successfully punctured the displaced air could be seen bubbling from the hole in the shell. When all the air was displaced the needle was quickly withdrawn, the aperture in the shell sealed with plaster of Paris and the egg returned to the incubator.

The eggs used for controls were subjected to the same experimental treatment save that sterile salt solution was injected instead of the thyroxin solution.

Stringent aseptic precautions were taken during the course of the operations.

The maximum amount of fluid that can be introduced into the air space of the egg at the third day of incubation was found to be in the neighbourhood of 2ccs., but there was considerable variation in the size of the air space and usually only about 1 cc. of fluid was injected; in the table the volume of fluid injected is not stated but the actual quantity of thyroxin injected is given.

In the early part of this experimentation, the dose of thyroxin given was calculated from the iodine content of the thyroid of an adult bird per gram of body weight. This was found to be approximately 0.00024 mgrms. of iodine per gram of body weight. On the basis of the approximate average weight of the eggs used (50 grams) an amount of thyroxin equivalent to 0.012 mgrms. of iodine was used. Since the monosodium salt of thyroxin is composed of 61% by weight of iodine, the dose of thyroxin to be injected on the basis calculated above is approximately 0.02 mgrms. As an extremely heavy mortality resulted from these early experiments some reduction in the dose was necessary. Solutions of the following strengths were made up for injection:-

1. 0.02 mgrms. of thyroxin per cc. of saline.
2. 0.016 " " " " " " "
3. 0.01 " " " " " " "
4. 0.002 " " " " " " "

Although these concentrations were productive of a relatively high death-rate in the injected eggs the dose was not further decreased as a maximum effect on the embryos was desired. The total number of eggs used in these experiments was 226. Of these 190 received injections of thyroxin while 36 received injections of saline only. That the number of controls

used was small compared to the number of eggs receiving the thyroxin was due to the fact that the preliminary experimentation showed that the death-rate was likely to be extremely high from these thyroxin injections, and, with only a limited number of eggs available, it was desired to obtain as many experimental embryos as possible.

The eggs used for the experiments were from a pen of Light Sussex hens mated with a Rhode Island Red cock (a sex-linked cross) so that the genetic sex of the embryo could be determined by macroscopical examination of the plumage.

On removal from the incubator for examination of the embryos at varying periods during incubation, namely the 10th, 11th, 16th or 17th days, the eggs were opened and identification of the sex by an examination of the plumage was made. The yolk was then removed and the embryos weighed. This was followed by a dissection and examination of the reproductive system after which the gonads were removed to a fixing fluid (Allen's modification of Bouin) for subsequent histological examination.

R E S U L T S.

It was found that out of a total of 190 eggs injected with thyroxin only 39 embryos survived the effect of the treatment and were alive at the time of examination. That the causal factor of this high mortality was the thyroxin and was not due to the experimental method employed was shown by the fact that the eggs used as controls, which underwent a precisely similar experimental treatment except that saline alone was injected, showed a very much lower death-rate. Of the 36 eggs injected as controls 29 survived until the time of examination. The death-rate in the thyroxin injected eggs was therefore 79.5% while that in the eggs injected with sterile saline was only 19.5%.

TABLE.

NO. of BATCH	NO. of days INCUBATED	EXPERIMENTAL MALES Weight in grams.	Dose mgrms. Thyroxin.	EXPERIMENTAL FEMALES. Weight in grams.	Dose mgrms. Thyroxin.	CONTROLS. Weight in grams (Males)	Dose in grams (females)
10	10	2.67	.016	2.92	.02	2.60	2.88
"	"	2.94	.01	2.77	.02	2.59	2.29
"	"			3.20	.02		
"	"			2.82	.02		
"	"			3.02	.016		
"	"			2.42	.016		
"	"			3.05	.01		
"	"			2.94	.01		
12	11	* 2.70	.016			3.8	3.4
"	"	4.40	.016				
13	"	4.60	.01	4.35	.01		3.5
"	"	3.10	.01	4.10	.005		4.1
"	"	4.75	.005	4.00	.005		
5	16	* 12.5	.016	*13.70	.002	16.5	15.35
"	"	18.0	.01	16.00	.01		17.00
"	"	20.0	.002	15.20	.01		19.20
"	"	15.8	.002	15.70	.002		
"	"			*13.15	.002		
8	"			*12.72	.01		
12	17	* 12.75	.01			19.6	22.05
"	"					22.1	
14	"	18.5	.0025	16.9	.005	19.2	24.50
14	"	* 14.0	.005			20.80	21.90
7	"	* 12.17	.02	*10.95	.016	20.75	21.64
"	"	19.27	.015			20.70	17.80
"	"	* 8.07	.01				21.50
11	"	* 9.92	.01				24.35
"	"	* 12.15	.o15			24.10	20.20
9	"			19.8	.01	17.65	22.20

* Embryos showing effect of Hyperthyroidism marked with an asterisk.

No deviation from the normal development of the reproductive system either in the embryos from thyroxin-injected eggs or in the control embryos was encountered. Further, the sex of the individual as determined by an internal examination of the embryo invariably agreed with the sex as determined by inspection of the down colouration.

The weights of the experimental and control embryos obtained are given in Table I., and although the numbers were not very large it will be readily seen that some of the chicks have been markedly affected by the thyroxin. This is not noticeable in those embryos examined in the second week of incubation.

The embryos which can be considered as showing symptoms of hyperthyroidism are confined to those examined on the 16th and 17th day of incubation (with possibly one embryo at the 11th day of incubation). It would thus seem that the effect of the thyroxin is not reflected in the growth-rate of the embryo until some point later than the 10th or 11th day of incubation. Examination of the embryos which did not survive the injection showed that there were two peaks of mortality, one soon after the injection and the other about the twelfth day of incubation.

The facts that no marked effect on the injected embryo is shown until at least the 11th day of incubation, and also that around this period there is a

decided rise in the mortality rate suggest that the embryo reacts to the thyroxin at a particular developmental stage. On the other hand it is obvious that such results would be also explicable if it could be shown that through physical interference the thyroxin did not become absorbed into the embryonic circulation until this time.

As there is apparently no direct correlation between the amount of thyroxin injected and the effect on the embryo it would seem that an appreciable amount is lost and is not available for absorption into the embryonic circulation. In this connection there are at least three sets of factors to be considered.

(1) The earliest stage at which the area vasculosa of the chick has extended sufficiently to bring it into close proximity to that part of the inner shell membrane underlying the air space.

(2) The permeability of the inner shell membrane and layer of albumin to the thyroxin.

(3) Evaporation of the fluid from the air space and deposition of thyroxin on the membrane.

It therefore becomes necessary to show that, if the favourable conditions for the absorption of the thyroxin depend on the extension of the area vasculosa over the surface of the inner shell membrane under the air space, these conditions are present before the end of the 6th day of incubation when differentiation of the gonads first can be satisfactorily demonstrated.

Examination of a number of eggs revealed the fact that at the 3rd day a small crescentic area of the vasculosa overlaps the inner membrane. This gradually increases until at the 6th day the conditions for absorption from the whole surface of the inner shell membrane are realised.

Preliminary experiments were made to determine approximately the rate of absorption of fluid through the inner shell membrane to the egg. Absorption was found to take place rapidly. Two and a quarter hours after the injection the air space was found to be nearly dry while the remaining injected eggs examined after a period of 24 hours were found to have completely absorbed the fluid. Although the conditions of incubation are not such that rapid evaporation of moisture from the egg is conceivable, it was thought profitable to determine approximately the rate of evaporation of fluid from the surface of the egg. For this experiment five eggs were "blown", 2 ccs. of saline were injected and the aperture in the shell then sealed. The eggs were placed in the incubator and removed after 24 hours and the volume of the unevaporated saline was determined. The loss by evaporation varied from 30-50%. Since nearly all the fluid in the air sac disappears within 2 hours of injection, the loss due to evaporation need not be considered here.

Histological examination of the gonads from

both the thyroxin-injected chick embryos and controls was made. There was no indication that the thyroxin affects in any way the normal process of differentiation of an indifferent gonad into ovary or testis.

In the female embryo which had received thyroxin, the canaliculisation of the medullary cords, the secondary proliferation of sex-cords from the germinal epithelium, and the formation of the so-called luteal tissue had proceeded exactly as in the gonads from control embryos.

Moreover, from an examination of the ovary of control embryos at the 16th or 17th day it was seen that the cells formed by the secondary proliferation had entered into the prophase of the first maturation division. Similarly the nuclei of the secondary sex-cords of the ovaries from thyroxin-injected embryos showed that the same stage of the meiotic prophase had been reached.

It is thus seen that far from inducing gross histological modifications in the structure of the ovary thyroxin in these doses and injected into the air space apparently does not even affect the rate at which the processes of differentiation occur.

No histological differences in structure between the testes from thyroxin injected males and control males were found.

D I S C U S S I O N.

The results of this experimentation show that it is possible by injecting thyroxin into the air space of the incubating egg to produce dwarfing of chick embryos, this dwarfing being due presumably to the raising of the metabolic rate. These results are similar to those of WILLIER who did not use thyroxin but implanted thyroid tissue from fowls on to the chorioallantoic membrane of the developing chick embryo. It is reasonable to accept his conclusions that such modifications as reduction in size and emaciation of the body are to be regarded as hyperthyroid symptoms and mean that metabolism was increased, more particularly an acceleration of catabolism over anabolism.

When the bearing of these results on the question of the relation between metabolism and sex-determination is examined, it is seen that neither in the experiments of WILLIER nor in these of the present series was this increased metabolism productive of any modifications in the processes of sexual differentiation. Assuming that male is to be distinguished from female by a higher metabolic rate, it is of course to be expected on the above hypothesis that this treatment would affect only those embryos which are genetic females producing a modification of the processes of sexual differentiation in the male direction . As the

grafts of the thyroid tissue were made by WILLIER at a time when the differentiation of the gonad had already proceeded some considerable way, it is perhaps not reasonable to expect such modifications to be produced by this technique. As the injections of thyroxin were made at a stage in the development of the embryo at which no demonstrable differentiation of the gonads had occurred, it might be deduced from the results of the present series of experiments on chick embryos that the theory that an increased metabolism tends toward the production of a high sex-ratio has not been upheld.

It cannot be said however that the results recorded here can lead as yet to any definite conclusion as to the validity of the hypothesis, for although the injections were made at the third day of incubation no effect on the embryo was observed until, at the earliest, the 11th day of incubation. That the effect of the injections does not take place until about this stage in incubation is also shown by the fact that there is a definite peak of mortality in the experimental embryos about the 12th day of incubation.

The present paper is of the nature of a preliminary report. It will be necessary to determine by further experimentation whether the apparently delayed action of the thyroxin is a specific characteristic of the substance itself, acting on the developing

chick only at a definite stage in development, or whether the results obtained were due to mechanical hindrances by which the absorption of the thyroxin into the embryonic circulation was not effected until some considerable time after the injection of the solution into the air space of the egg.

S U M M A R Y.

1. An attempt was made to determine in the developing fowl embryo whether an increase in the metabolic rate would lead to a modification of the processes of sexual differentiation.

2. The method employed to raise the metabolic rate was the injection of the active principle of the thyroid gland into the air space of the incubating egg.

3. The injections were made during the third day of incubation, that is, at a time before the differen-:tiation of the gonads in the two sexes can be demonstrated.

4. Of the 190 eggs injected with thyroxin, 39 embryos survived of which 12 were markedly affected, reduced size of embryos being an indication of a marked hyper-thyroidism which has resulted in an increase in the metabolic rate.

5. No effect on the processes of sexual differentiation in either sex was found.

6. The effect of the injections was not apparent before the eleventh day. It will be attempted in a further series of experiments to determine whether this is

a/

a specific reaction of the thyroxin or whether it is due to mechanical hindrances to the absorption of the thyroxin into the fœtal circulation until a considerable time after the injection.

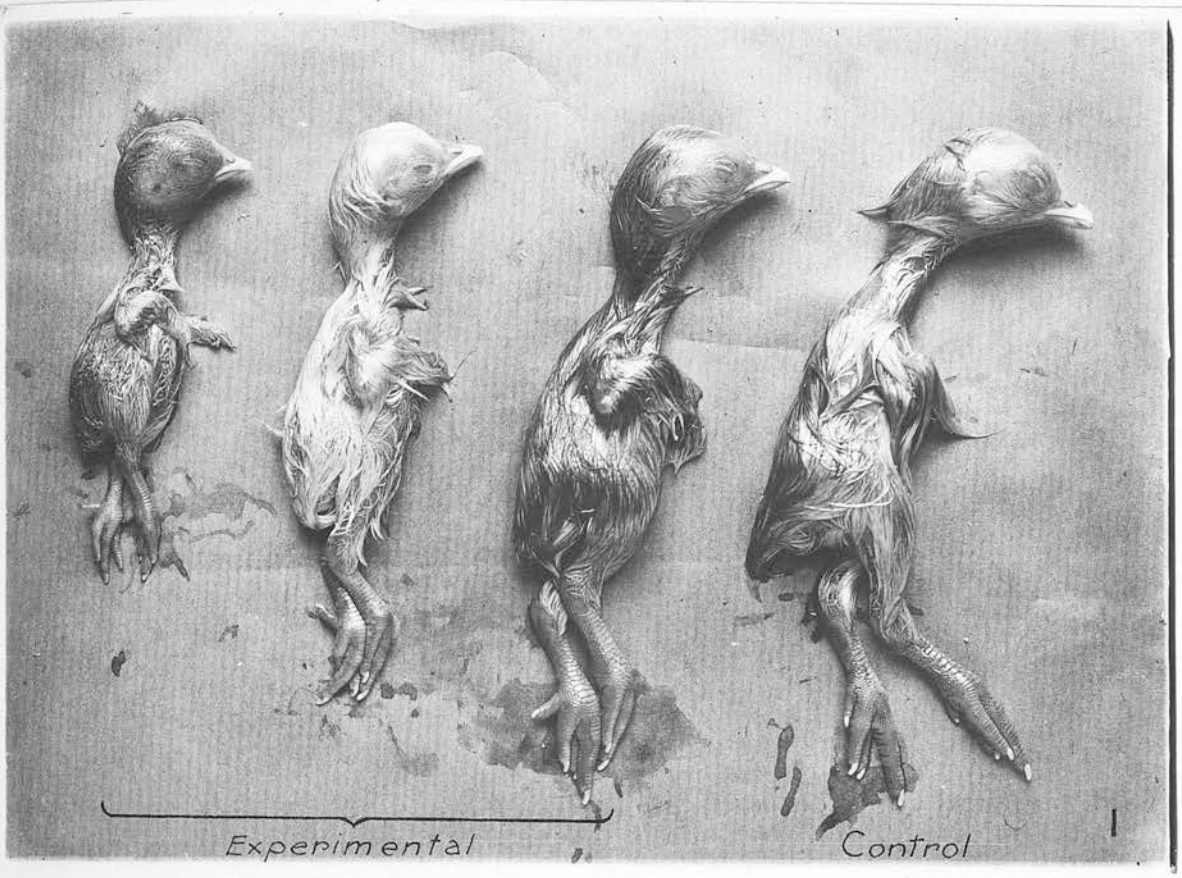
7. These experiments can not at present lead to any definite conclusion as to the relation between increased metabolism and the processes of sexual differentiation.

FIG. I.

Photograch of three embryonic chicks at 17th day of incubation which received thyroxin injection along with one control which received no thyroxin. The treated ones show reduction in size due to high metabolic rate of growth. From batch 7.

FIG. II.

Photograph of two embryonic chicks at 17th day of incubation which received thyroxin injection along with one control which received no thyroxin. From batch 11.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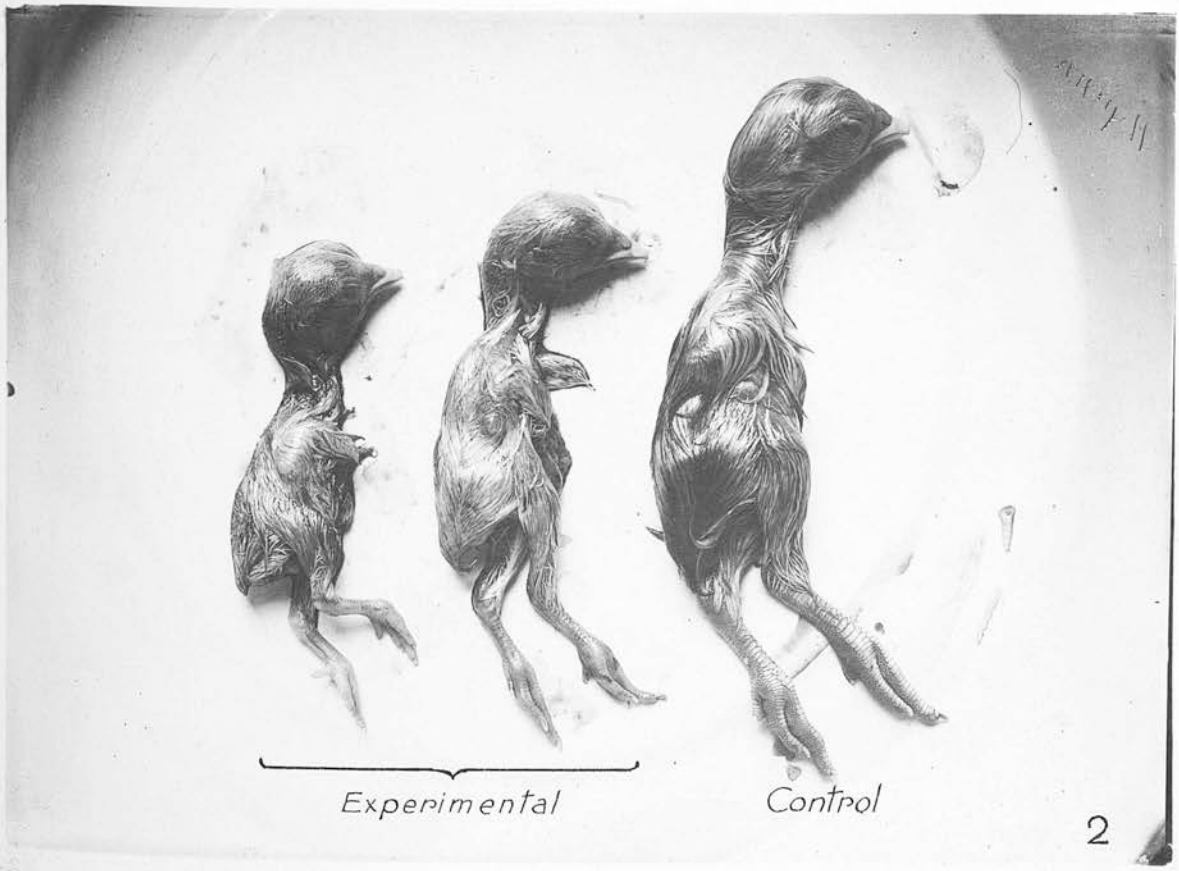
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A STUDY ON THE PIGMENTATION OF THE HIMALAYAN RABBIT *

by

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* Has been accepted for publication in the Proceedings of the Royal Physical Society Edinburgh.

A STUDY on the PIGMENTATION of the HIMALAYAN RABBIT.

Attention has recently been directed to the question of pigmentation in the Himalayan Rabbit, this breed being white except for the legs, tail, nose and ears, which are black. It has been found that the exhibition of pigment may be controlled by alterations in temperature, SCHULTZ (1915), for example, having shown that white hair was replaced by pigmented, under the influence of a lowered temperature. ILJIN (1921), studying the differential response of certain skin areas to this altered environment concluded that each part exhibited a different threshold of response. According to the work of DURHAM (1904), GORTNER (1911-1912), and PRIZBRAM, DEMBOWSKI and BREECHER (1921), animal pigment is a melanin produced by the action of an oxydase (tyrosinase) on a colourless chromogen (tyrosine).

The question arises as to whether the pigment under consideration is the result of the action of these two factors, or due to some other agency, and it has been investigated to some extent by LAURA KAUFMAN (1923) who obtained pigmented hair in place/

place of white by injecting tyrosinase extract (in one case out of twenty-eight) and by injections of H_2O_2 (in two cases out of ten). The results were more definite when a dilute solution of NaOH was added to extracts of skin of young Himalayans, the extracts turning dark on incubation. In view of the fact that dioxyphenylalanin becomes dark on addition of alkali, she suggests that the chromogen present in the Himalayan rabbit may be of similar nature to that substance.

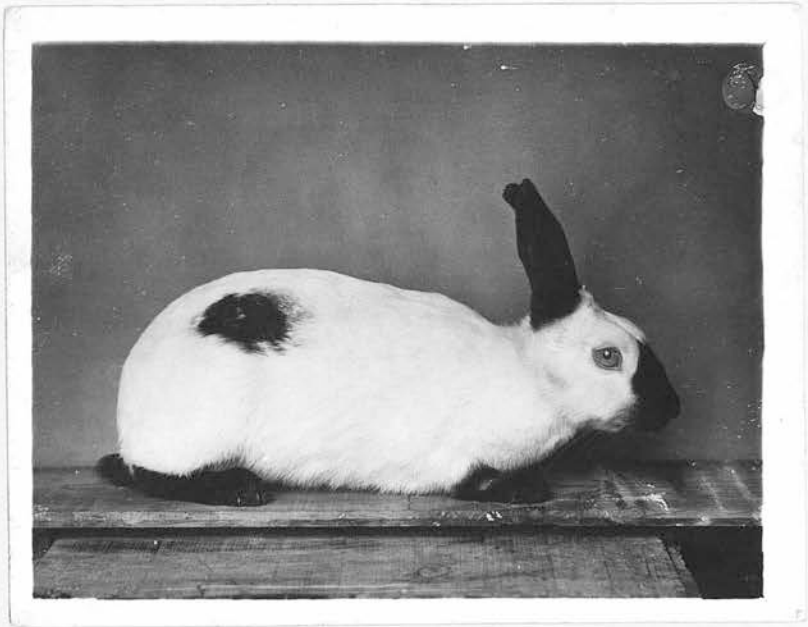
There appears to be no explanation of the pigment distribution in the Himalayan nor any determining factor except temperature, and these experiments were undertaken with a view to elucidating the problem.

EFFECT on ADULT RABBITS.

The material used was pure-bred Himalayans. In order to test their susceptibility to the Schultz reaction, a portion of the hair from the sides of these rabbits was shaved off, and the hair which grew subsequently was black, though the production of pigment varied in time and degree. The temperature/

FIG. I.

A patch of black hair grown on the back of a Himalayan adult rabbit after the part had been shaved and the rabbit exposed to low temperature.



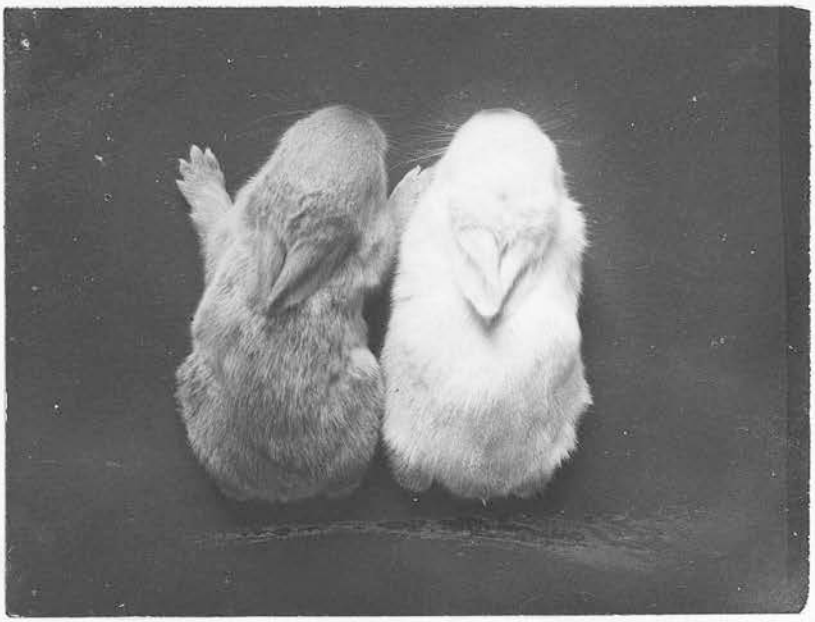
temperature in the rabbitry from the time when the skin was shaved until the appearance of the black hair was 5° - 9.5°C. After shaving the skin is white, but later black patches appear on it. These gradually become more numerous, until ultimately the shaved area is entirely black, and finally tufts of black hair appear, and in their turn gradually spread over the black surface until the hairy covering is reconstituted. After attaining a certain length the new hair begins to grow lighter and becoming white, the inference being that the protection afforded by the hair raises the skin temperature sufficiently to inhibit the further production of pigment. (fig. 1).

EFFECTS on NEW-BORN HIMALAYANS.

The baby coat of the Himalayan is white; the pattern only develops gradually after a few weeks when the young rabbits move about more freely in the cage. It was thought desirable to determine the effect of low temperature upon these young rabbits/

FIG. II.

A Himalayan rabbit, one day old, sponged with cold water for 10 minutes for 2 days grew a black coat in contrast to its untreated control from the same litter. Photographed when 2½ weeks old.



rabbits at the stage when the pigment was as yet undeveloped. Accordingly two were taken from one litter and exposed to the air in the rabbitry outside the nest for ten minutes, a temperature of 17°C. They were then returned to the nest, and the experiment was repeated in a similar manner on the following day, with the result that in one to two days the skin began to darken, and this was followed by the growth of dark hair which subsequently became lighter as in the first experiment upon adults.

A second pair were taken from another litter oneday after birth and were sponged with water at a temperature of 17°C, the temperature of the rabbitry at that time being 20.5°C., and that of the rabbit's back about 35°C. An ordinary thermometer was used, and the sponging was carried out on two successive days, the rabbits being out of the nests for ten minutes on each occasion. The same changes were observed and the alteration in colour is shown in FIG. 2., where one of the rabbits subjected to sponging has been photographed with a control from the same litter.

Dark/

Dark hair can be produced in rabbits one to two days old by exposure to a temperature of 12 - 17°C, but this temperature is not sufficiently low to have the same effect in rabbits of $2\frac{1}{2}$ weeks from which it is concluded that susceptibility to temperature is more marked in newly-born rabbits.

EFFECT of INJECTION and SKIN EXTRACTS.

In order to discover the nature of the chromogen involved in the process of pigment formation, the skin of a Himalayan rabbit one to two days old was extracted by ONSLOW'S (1925) method with Kieselguhr and chloroform water. To equal volumes of the extract in test tubes tyrosine extract was added, with and without H_2O_2, and to others NaOH of varying dilutions; the tubes were then stoppered and incubated at 37°C. None of the tubes showed any sign of darkening even after prolonged incubation, although KAUFMAN found positive darkening of the extract with NaOH. To further test the action of NaOH on the skin, pieces of skin from/

from newly-born Himalayans were taken immediately after death and placed in test tubes with NaOH of various strengths, and a few drops of $CHCl_3$ as a preservative, a few being filled with distilled water to serve as controls. Half the tubes were exposed to room temperature, and half were incubated at 37°C, but in neither series was any darkening observed. Some pieces of skin were then incubated in tyrosine suspension with and without H_2O_2, but without any result.

If the effect of low temperature has any influence upon the plasma reaction in the living animal, as argued by KAUFMAN, and this in turn upon the production of pigment, the application of alkali (e.g., NaOH solution) to the skin should produce black hair. To test this hypothesis the hair from the side, a few cms. square, was removed by shaving and a few drops of very diluted NaOH solution was injected into the bare skin in four or five places, but the result was negative. In another series the hair was shaved on one side and the skin was soaked with a solution of NaOH dilution for several days, but/

confined to the hair root and appearing to have its origin in the epithelial cells of the bulb, the epithelial cells alone showing pigmentation. In this the skin of the young Himalayan is comparable to that of a rabbit from a black strain, the latter being black from birth. In many sections it was noted that the portion of hair immediately beneath the skin surface was not pigmented, the pigment being confined to the bulbs. This would seem to indicate that during the growth of the upper portion pigment was still absent from the bulb, and would only advance towards the skin surface at a late period. Under the stimulus of low temperature, pigment is formed in the bulb and is included in the growing hair, as observed by DRY (1926), in the pigmented hair of the mouse. When the stimulus ceases the production of pigment is arrested in the bulb but the previously formed pigment grows up into the medulla of the hair leaving the bulb devoid of it. This distinction between the pigmentation in Himalayan and black breeds is that the length of the pigmented

hair/

hair in the former is dependent upon its length and intensity of the stimulus applied, whereas in the black rabbit the hair is pigmented throughout and is not influenced by environment. The depth of colour in Himalayan rabbits is variable, depending upon the number of pigment granules present, and this in turn is conditioned by the intensity of the stimulus applied.

Discussion/

D I S C U S S I O N.

It has been shown that white skin areas in the Himalayan rabbit are capable of pigment production without introducing into the skin any chromogen or oxidising agent. ILJIN was able to grow white in place of black hair on the nose and ear by raising the temperature. The Experiments described in this paper show that the entire skin surface of the Himalayan rabbit may be made to produce dark hair by lowering the temperature from which it is evident that this is a self-coloured breed, the absence or exhibition of pigment depending upon the temperature to which the animal is exposed.

The reason for the distribution of pigment in the animal remains to be considered. ILJIN'S-statement that different body areas showed varying thresholds of response is already referred to; he suggests that the cause of the differences exhibited by different regions lies in the underlying/

FIG. III.

Photograph of a 4 day old Dutch rabbit. The future pattern of the adult is visible even at this stage, which is unlike that of the young Himalayan rabbit. In this breed the factors for pigment production are restricted to the parts showing pigmentation.

underlying differences in subcutaneous regional blood supply. The lower threshold of irritation of the extremities is to be explained by reference to a restricted blood supply consequent upon this constriction in response to external cold. KAUFMAN found a difference of temperature between the pigmented and unpigmented parts, using a mercury thermometer.

The pattern of the Himalayan and Dutch rabbit differs in that the pigment of the Dutch rabbit is independent of temperature whereas that of the Himalayan is conditioned by such alterations in environment. The patterns of Dutch and English rabbits are present from birth, while that of the Himalayan rabbit is developed at a later period. KAUFMAN'S result in pigment production through the application of NaOH has not been confirmed in this series of experiments, although the alkali was applied in various ways to eliminate all source of error. SCHULTZ also failed to confirm her result.

Extracts from the skin of new-born Himalayans showed no pigment formation with tyrosine this/

this might be due to the absence of an oxydase in the skin at the time of the examination. KAUFMAN was able to produce black hair in three cases by injecting tyrosinase and H_2O_2, which suggests that the skin is capable of producing pigment on addition of an oxydase. The problem remains concerning the mechanism involved in pigment formation at low temperatures. It should be noted that the skin of a new born Himalayan which has been killed and subjected to cold sponging or other exposure to low temperature does not turn dark as in the case of the living animal.

According to SCHULTZ, unpigmented skin gives a negative dopa-reaction but the skin during the stage of active pigment formation gives a positive dopa-reaction, a negative reaction being again obtained when active pigment production ceases. This would indicate the presence of some substance in the skin during pigment formation which is absent at other times. PRIZIBRAM, DEMBOWSKI & BRECHER found that both tyrosine and "dopa" became black/

black under the action of tyrosinase. The effect of low temperature on pigment production appears to be entirely local since only those parts of the skin actually exposed to low temperature produce pigment subsequently.

In the light of this evidence one may put forward the hypothesis that under the influence of low temperature the living epithelial cells of the hair follicles are stimulated to secrete some oxidising enzyme which leads to the formation of pigment through reaction with a chromogen. The fact that the skin of young Himalayans does not become dark after death supports the suggestion, since death would inhibit the production of such a ferment. It might be stated in this connection that most of the hair of Chinchilla rabbits shows pigmented and unpigmented bands which is perhaps due to temporary cessations in pigment production.

I wish to express my gratitude to Dr Crew for the help and encouragement he has given me through the course of this investigation.

S U M M A R Y .

1. Adult Himalayan rabbits can be made to grow pigmented hair in parts which are normally white.

2. The skin of new born Himalayan rabbits is more sensitive to temperature than that of adults, susceptibility being markedly decreased even at the age of $2\frac{1}{2}$ weeks.

3. The dark appearance of the skin prior to the growth of black hair is due to the presence of pigment granules in the bulb of the growing hair.

4. The pigment originates in the epithelial cells of the bulb of the hair follicles.

5. The degree pigmentation depends upon the intensity of the stimulus applied i.e., degree of cold applied and length of exposure.

6./

6. The comparatively lighter colour of the pigmented hair as compared with those areas which are normally black, is due to the smaller number of pigment granules.

7. The formation of pigment in the bulb stops coincidently with cessation of the stimulus.

8. The skin of new born Himalayans after death does not show any pigmentation under low temperatures.

9. Solutions of NaOH applied by various methods to the skin and to skin extracts does not produce any blackening.

10. The distribution of pigment in the Himalayan is due to the relatively low temperature of the extremities.

11. Low temperatures may influence pigment formation by stimulating the epithelial cells of the hair follicles to secrete an oxidising ferment.

PLATE I.

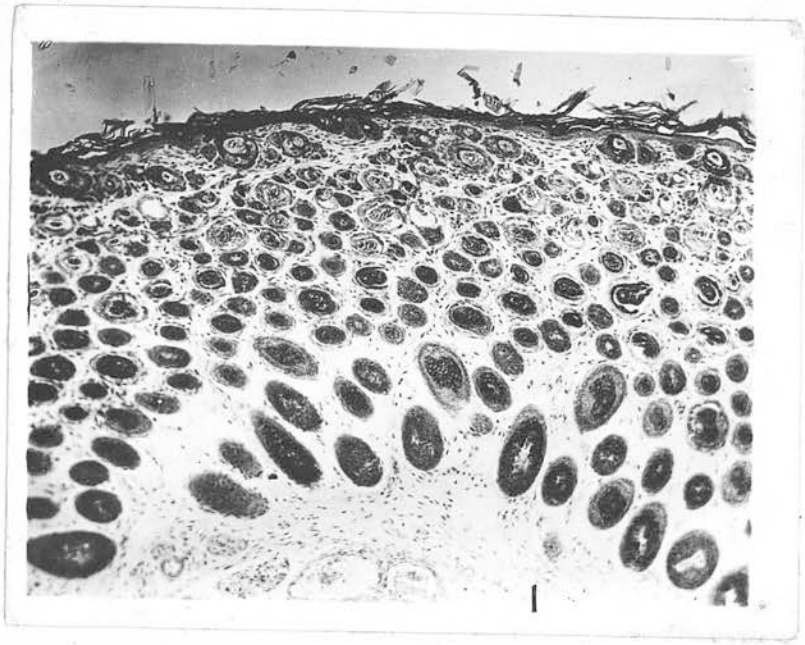
MICRO-PHOTOGRAPH X 90.

Transverse section of the skin of the tail of a 4 days old Himalayan rabbit which has been subjected to low temperature. Because the hairs grow at a very small angle with the surface of the skin, the hairs below the surface of the skin being cut obliquely. There is comparatively small amount of pigment in sectioned hairs that also is confined to lower regions.

PLATE II.

MICRO-PHOTOGRAPH X 90.

Transverse section of the skin of the tail of a 4 days old black rabbit. The hairs being sectioned more or less transversely - abundant black pigment in the sectioned hairs in contrast to that in the section from the Himalayan young rabbit. The pigment is present in all portions of the hair.



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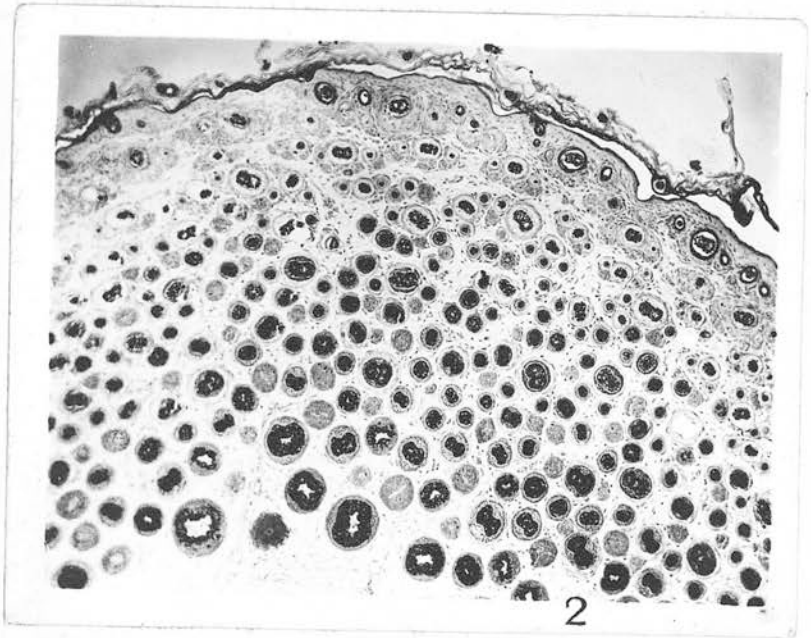

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PLATE III.

MICRO-PHOTOGRAPH. X 160.

Longitudinal section of a hair with root of a young black rabbit. Showing the pigment in the follicle and the hair.

PLATE IV.

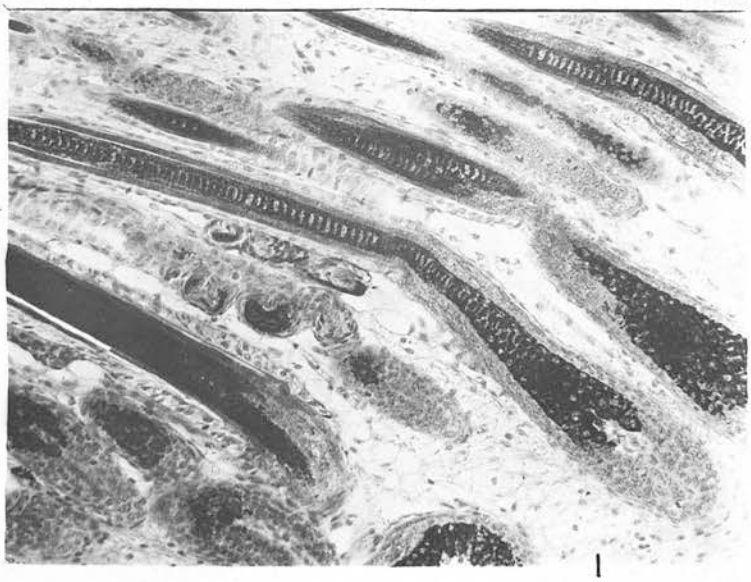
MICRO-PHOTOGRAPH X370.

Longitudinal section of a hair follicle of a Himalayan young rabbit which has been subjected to low temperature. The pigment is confined to the epithelial cells of the follicles.

PLATE V.

MICRO-PHOTOGRAPH X 370.

Longitudinal section of a hair follicle of a 4 days old Himalayan rabbit subjected to low temperature. There is hardly any pigment in the root, but there is a little pigment at the neck showing the passage of the pigment to the hair.



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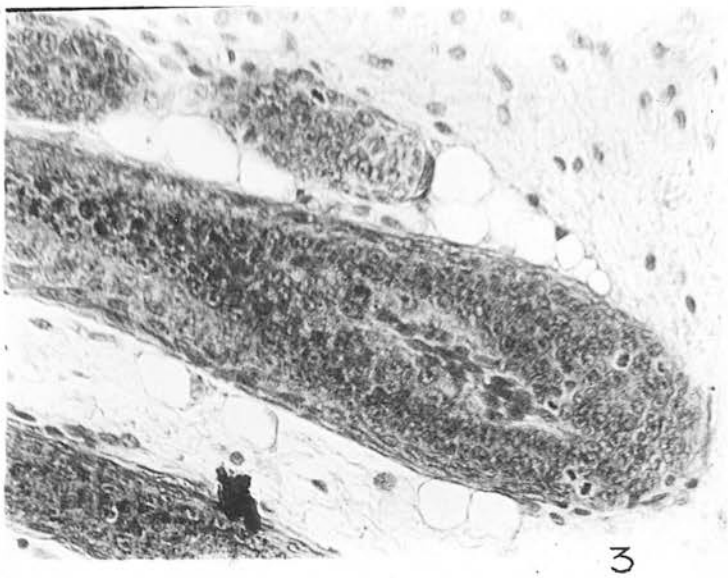

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FIG. VI.

Photograph (X $1\frac{3}{4}$) of hairs of a Himalayan rabbit. The top portion is black and the lower portion is white. These black hairs were grown on the back under the influence of low temperature, where normally white hairs grew. The protection caused by the black portion of the hair raised the temperature of the surface of the skin, and led to the cessation of pigment formation.

FIG. VII.

Photograph of hairs of a Chinchilla rabbit showing pigmented and unpigmented bands.

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Age and Quality of Offspring

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AGE AND QUALITY OF OFFSPRING

The Effect of Age of Parents on the Quality of Offspring in Cattle

A. C. CHAUDHURI

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SOME work has been done on the relationship of the age of the dam to fertility and fecundity in different animals, but the literature on the relation of the age of the parents and the quality of the offspring does not seem to be satisfactory. Many breeders doubt whether the offspring of aged parents is as good as that of young ones. The greatest age at which a cow can breed is still somewhat doubtful. The Aberdeen-Angus foundation cow, "Old Grannie," produced a calf in her twenty-ninth year. Jones and Rouse[4] record a cow calving at the age of thirty-three, and two multiple births at the age of twenty-five in the American Hereford and Aberdeen-Angus herd books respectively. In the horse, Wood[6] records a thoroughbred mare breeding at thirty-three, and two more at thirty, while in the sheep Pearl[5] has noted a remarkable ewe which produced a lamb in her seventeenth year. These are merely some figures to show the limit: the average age at which a cow stops breeding is considerably lower. There is no record as to the relative quality of the offspring of a particular individual according to age.

In order to present some data concerning this, the prizewinners in the Shorthorn classes at the Highland and Agricultural Society's show (the leading show in Scotland) and their parents were studied, the ages of the parents being traced in Coates' Herd Book. The lists of premiums recorded were from 1902-1925, excluding four years when no shows were held, thus leaving records of over twenty shows. Records were made of the first five prizewinners of each class excepting those in which there were only a few entries, and these were eliminated. To each first prizewinner five points were awarded; to the second, four; and so on, the fifth getting one. The date of birth of these winners were recorded, also the ages of their dams and sires at that time. As, for various reasons, most animals are not allowed to live as long as they are capable of breeding, it was thought desirable to compare the number of points gained by cows of a certain age as parents of prizewinning offspring, with the number of cows calving at that age in a similarly controlled population. For this the ages were ascertained of a number of the cows which calved in the age periods, between volumes 58 and 70, both inclusive, of Coates' Herd Book. These volumes cover the greater part of the population from which the winners came. The age of the dam of every hundredth birth was recorded, amounting to 2,078 births.

The data thus collected are tabulated in Table I.

While the figures cannot be regarded as in any way significant, or definite, they are perhaps suggestive. From columns 1 and 2 it may be noted that the maximum number of prizewinning offspring belong to dams who calved at three and four years, but this is balanced by the fact that Shorthorn cows are more regularly served to calve at that age (column 4) and indeed the number of points gained per hundred cows at that age differs little from any other age (column 5). The average number of points per hundred cows increases perhaps with age, but this, if it is so, cannot be regarded as significant, for

it is only natural that breeders should retain in their herds for a rather longer period cows which they recognize as giving rise to offspring above the average. A study of these figures also shows that with an advance in age more and more breeding cows are disposed of, the disposal starting in the third year but not severely until the fourth. From the fourth to the seventh year there is a heavy reduction, then a slight easing off till the ninth, after which it is somewhat more gradual.

As regards the age of the sire on the quality of the offspring, results are somewhat similar. Six more winners are included in this study, it having been impossible to trace their dams. It is interesting to note that the greatest number of prizewinners are produced by sires which were two years old when their prizewinning progeny were born. This is logical, and it shows that a good many breeders prefer to use young bulls, which is in accord with the common practice in Shorthorn herds in Scotland.

Donaldson[2], working with rats, finds that the birth weight increases with the increasing age of the dam. This is due rather to an environmental cause than to a genetic one. Eckles and Palmer[3] state that neither the percentage composition of the milk nor the physical and chemical constants of milk fat from aged dairy cows show any abnormalities attributable to old age. They further record that butter made from the milk of a cow nineteen years old was pronounced to be of excellent quality. Allen[1], working on the ages of the sires and dams of cows under three age groups according to their seven days butter-fat production (Advanced Registry, American Holstein Friesian), concluded that the parentage of superior or high producing dairy cows is no older than the parentage of

TABLE 1—CLASSIFICATION OF PRIZE WINNING SHORTHORN CATTLE ACCORDING TO THE AGE OF PARENTS

	According to the Age of Dam							According to the Age of Sire			
	1	2	3	4	5	6	7	8	9	10	11
Age of Dam or Sire	No. of winners	No. of points gained by winners	No. of points per winner	No. of cows calving in age period in hundreds	No. of points per hundred cows calving	Per cent. of total points	Per cent. of total no. of cows calving	No. of winners	No. of points gained by winners	No. of points per winner	Per cent. of total points
2	51	164	3.21	191	0.85	9.66	9.19	183	550	3.00	32.04
3	95	315	3.31	416	0.75	18.57	20.02	153	480	3.13	27.96
4	100	299	2.99	361	0.78	17.62	18.34	88	278	3.15	16.19
5	71	212	2.98	291	0.72	12.49	14.00	44	122	2.77	7.11
6	57	185	3.24	234	0.79	10.90	11.26	37	121	3.27	7.05
7	46	150	3.25	173	0.86	8.84	8.33	27	97	3.59	5.65
8	43	131	3.04	138	0.94	7.72	6.65	8	29	3.62	1.68
9	31	103	3.32	102	1.00	6.07	4.90	7	22	3.14	1.28
10	15	39	2.60	52	0.75	2.30	2.50	6	13	2.16	0.75
11	15	35	2.33	36	0.96	2.06	1.74	2	5	2.50	0.29
12	10	20	2.00	32	0.62	1.18	1.54				
13	7	21	3.00	14	1.50	1.23	0.68				
14	3	4	1.33	10	0.40	0.24	0.48				
15	1	3	3.00	5	0.60	0.19	0.24				
16	3	11	3.66	1	11.00	0.64	0.04				
17	-	-	-	2	-	-	0.09				
18	1	5	5.00	-	-	0.29	-				
Total	549	1,697		2,078		100	100	555	1,717		100

comparatively inferior or low producing cows.

These results are in accord with those presented in this paper. While the figures herein shown are only suggestive and in no way conclusive, nevertheless there is nothing that can be deducted from them to show that there is either a decrease or increase of quality according to the age of the parents. This result is in accord with accepted genetical theories.

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